



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of

**MUTSAERS et al**

Atty. Ref.: **BHD-4662-212**

Serial No. **10/584,921**

Group: **1619**

Filed: **June 29, 2006**

Examiner: **SMITH, Chaim A.**

For: **NOVEL PROCESS FOR ENZYMATIC BLEACHING OF FOOD PRODUCTS**

\* \* \* \* \*

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION PURSUANT TO RULE 132**

Sir:

The undersigned, P. Langerweld, hereby declares and states that:

1. I am and for all times relevant to the facts stated herein have been employed by DSM Gist Services B.V. and am familiar with the invention described and claimed in the above-identified US patent application.
2. Under my direction and control, the following experiments were conducted so as to demonstrate the effects of the presently claimed invention on whitening annatto-containing whey. Specifically, the following Examples 1 and 2 were conducted.

**Example 1**

Bleaching experiments were performed in fresh whey from a cheddar cheese production on a pilot scale.

To test the bleach performance of an enzyme for which annatto is a substrate (MaxiBright™ enzyme bleaching agent commercially available from DSM N.V., a microbially expressed enzyme cloned from *Marasmius scorodonius* extract), 300 ppm of annatto was added to the cheese milk.

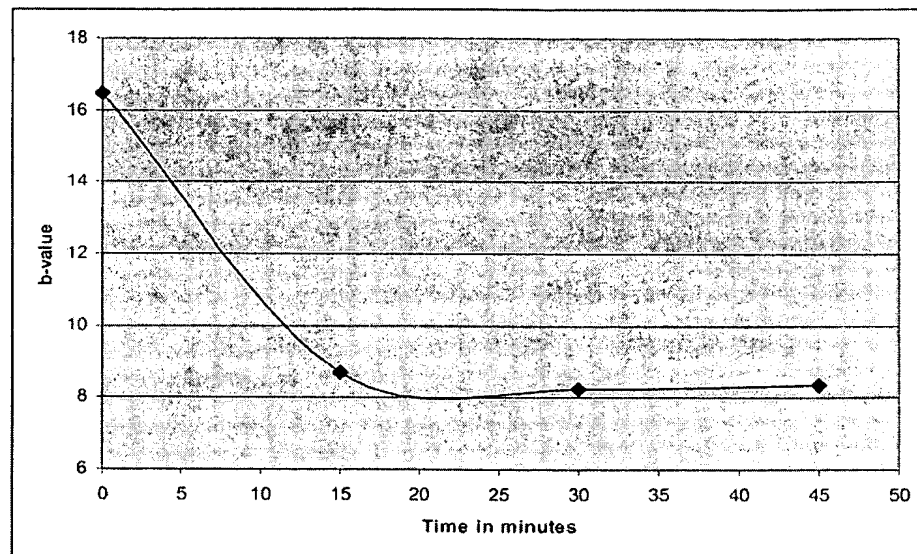
The bleaching experiment was done on a 100 ml scale under the following experimental conditions were:

- Temperature: 20°C
- MaxiBright™ enzyme DBL.GRZ.0848: 1 DBLU/ml whey. (The MaxiBright™ enzyme activity is expressed in DBLU (Dairy BLEaching Units). One DBLU is defined as the amount of enzyme that oxidizes 1 µmol ABTS per minute under conditions of the test (37°C, pH 3.50))
- H<sub>2</sub>O<sub>2</sub> : H<sub>2</sub>O<sub>2</sub> was added to the whey (final H<sub>2</sub>O<sub>2</sub> concentration in the whey 0.4 mM)
- pH: pH of the whey was 6.6

The colour of the whey was measured by means of X-Rite® 968 reflection spectrophotometer as described on page 5, lines 14-20 of the above-identified application where the following 3 components are determined: L=black/white, a=green/red, b=yellow/blue. It appeared that component b is most changeable and sensitive for yellow colour of whey coloured with annatto and therefore was used to determine the bleaching efficiency of the enzyme.

## Results

The results of whey bleaching are presented in Figure 1 where the b-value colour component (y-axis) as measured versus time (x-axis).



### Example 2

Bleaching experiments were performed in WPC80 powder dissolved in fresh water.

To test the bleaching performance, 300 ppm annatto was added to the WPC80 solution.

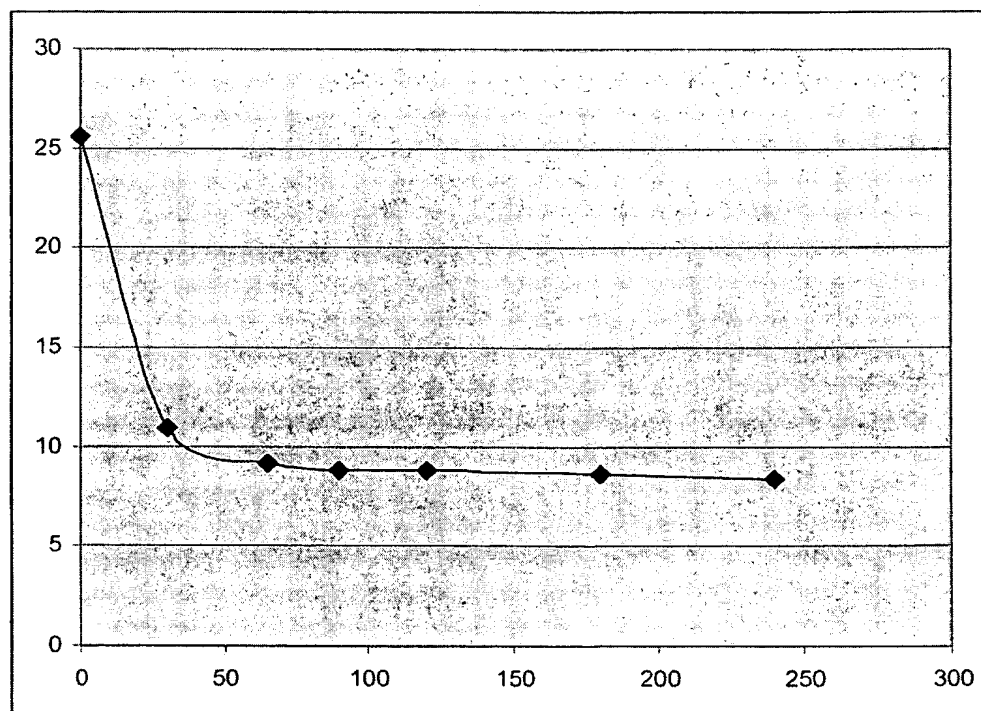
The bleaching was done on 50 ml. scale and experimental conditions were:

- Temperature: 23°C

- MaxiBright™ enzyme formulation 410131601: 2.25 DBLU/ml.
- $\text{H}_2\text{O}_2$  :  $\text{H}_2\text{O}_2$  was added to the WPC80 solution (final  $\text{H}_2\text{O}_2$  concentration in the whey 0.8 mM)
- pH: 5.9
- Dry matter: 16.7%

### Results

The results of whey bleaching are presented in Figure 2 where b-value colour component (y-axis) was measured versus time (x-axis).

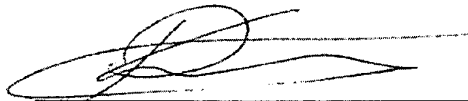


3. As can be seen from the graphs of Examples 1 and 2 above, annatto-containing whey can be substantially whitened by the direct conversion of the enzyme.
4. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully Submitted,

18-8-2011

Date Signed

A handwritten signature in black ink, consisting of a large, stylized 'D' followed by a horizontal line and a flourish.